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by

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I. INTRODUCTION

Since the demonstration by BLOCH and RITTENBERG in 1942 that all the carbon atoms necessary for cholesterol biosynthesis were provided from acetate, CALDWELL, LYNEN, WIELAND, et al. have advocated recently HMG-CoA cycle (β Hydroxy-β methylglutaryl-CoA cycle) and many other investigators have elucidated the details in the biosynthetic pathways of cholesterol. In addition, degradation of cholesterol has been also investigated. It is now generally accepted that cholesterol is metabolized to bile acids, sex hormones and adrenocortical hormones.

In our laboratory, HIKASA et al. investigated the various specific effects of essential fatty acids (EFA) upon cholesterol metabolism over longer period. They demonstrated that liver, adrenals and heart contained much EFA more than did the other organs in the experimental animals and human subjects. TAMAKI, ISHIMARU, KUMANO, FUKUDA and MURAOKA demonstrated in adrenals of rats, that EFA esterified with cholesterol, especially tetraenoic acids had an important influence upon adrenocortical capacity, by affecting directly cholesterol degradation to glucocorticoids. HIRANO, MARUYAMA and YOSHINAGA have recently demonstrated that the degradation of cholesterol to bile acids might also be affected by EFA in liver, presumably in the same way as in adrenals, before reviewing the various factors which increased and decreased cholesterol metabolism.

The experimental hypothesis was established that EFA-deficiency and/or metabolic disturbances in EFA due to pyridoxine deficiency and/or a decrease of the pyridoxine activity, affected directly cholesterol degradation to bile acids and biosynthesis of lecithin, leading to the decreased biliary excretion of bile acids and lecithin which in turn weakened the capacity of bile to keep cholesterol in solution so that cholesterol began to crystallize, precipitate and then cholesterol stones occurred.

In the former experiments, however, albino rats were always used, but they have no gallbladder. Hence, the present study was designed in part for the purpose of the establishment of our experimental hypothesis and in part for the experimental production of gallstones and for demonstration of some important inducements affecting gallstone formation, especially cholesterol stone formation.

II. EXPERIMENTAL ANIMALS AND EXPERIMENTAL METHODS

A. Experimental animals

The young golden hamsters (Mesocicetus auratus) were used from our own stock colony. During weaning and until the beginning of the experimental feeding period, they

had received a rat chow diet (produced by NIPPON HAIGOSHIRYO K. K.) and water ad libitum. They were distributed into groups and housed when their body weight had reached 30 to 40 g.

The various synthetic diets used in the present study were presented in Table 1. The maximal feeding period was 13 weeks

The first 10 groups, each of which was consisted of 10 to 20 hamsters (males and females were nearly equally divided), were used in order to demonstrate the incidence and characteristics of gallstones in relation to variations of the dietary compositions. The second 10 groups (each group contained 10 to 20 animals) were used for determination of bile acids, cholesterol and phospholipids in the drainaged bile with respect to difference between carbohydrates and/or fats added into the various synthetic diets. The third 5 groups, each of which also was consisted of 10 to 20 animals, were put to use for examing the specific effects of pyridoxine and fats upon the biliary excretion of bile acids, cholesterol and phospholipids. The animals (Group 26) received a rat chow diet were used for control.

B. Experimental methods

1. Establishment of bile fistula and collection of bile

After the intraperitoneal injection of 0.5% nembutal (mainly 0.1 ml per 100 g of body weight), the best exposure of gallbladder and bile ducts was obtained by making the upper middle incision as high as possible toward the thorax. Before intubation the common bile duct was ligated just below the bifurcation of the cystic bile duct in order to avoid the regurgitation of pancreatic juice. The fundus of the gallbladder was grasped with a pincette and elevated well upward to be advantageous in inserting a polyethylene tube and in bringing the content of the gallbladder and bile ducts into view. The fundus of the gallbladder was then opened with a scissor. When the content of the organs was removed completely by means of gentle pressing with gauze, a small polyethylene tube was inserted in the gallbladder so that it projected through the neck of the gallbladder into the ductus cysticus. The fundus was then closed tightly by means of a single ligature. After the wound was closed, the distal part of the polyethylene tube was conducted subcutaneously and placed in the small glass bottle fixed on the back of the animals. The essential requirement of intubation for collection of hepatic bile is that it must eliminate the contamination of pancreatic juice, excluding the many harmful actions of the gallbladder per se. This was accomplished by means of intubation via gallbladder and ligation of the common bile duct. The drainaged bile collected for 24 hours was analysed immediately for cholesterol, bile acids and phospholipids.

2. Extraction and determination of bile acids

An aliquot of the drainaged bile was added with 5 volumes of absolute ethanol, and was then heated on the steam bath for 30 minutes. After cooling the solution was filtered by glass filter. The ethanol solution was extracted twice or thrice with 20 ml of petroleum ether in order to remove neutral lipids. The rest of the petroleum ether extract was evaporated on the steam bath, dissolved into 5 ml of NaOH water solution, and finally autoclaved at 120°C for 2 hours.

After autoclaving the hydrolysates were acidified with 3 N HCl and extracted four

Table 1 Composition of diets

Group No.	1	2	3	4	5	6	7	8	9	10
Casein, crude	16.0	16.0		20.0	25.0	20.0	20.0	20.0	20.0	20.0
Casein, low vitamins			16.0							
Salt mixture[1]	3.0	3.0	3.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vit. mixture[2] No. 1.	0.5									
Vit. mixture[3] No. 2.		0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit. mixture[4] No. 3.			0.5							
Choline chloride	0.2	0.2	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Potato starch	80.3	80.3	65.3							
Sucrose				73.0	68.0	72.0		63.0	63.0	66.0
Glucose							72.0			
Sesame oil[5]								10.0		
Lard[6]			15.0						10.0	5.0
Cod liver oil[7]										2.0
C. M. C.[8]				1.0	1.0	2.0	2.0	1.0	1.0	1.0
Group No.	11	12	13	14	15	16	17	18	19	20
Casein, crude	20.0			20.0	20.0	20.0	20.0	20.0	92.0	87.0
Casein, low vitamins		20.0	20.0							
Salt mixture	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vit. mixture No. 2.	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit. mixture No. 3.		0.5	0.5							
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Soluble starch	74.0	74.0	74.0							
Dextrin							72.0	67.0		
Sucrose				72.0						
Glucose					72.0					
Lactose						72.0				
Sesame oil								5.0		5.0
C. M. C.				2.0	2.0	2.0	2.0	2.0	2.0	2.0
Group No.	21	22	23	24	25					
Casein, crude	20.0		20.0		20.0					
Casein, low vitamins		20.0		20.0						
Salt mixture	5.0	5.0	5.0	5.0	5.0					
Vit. mixture No. 2.	1.0		1.0		1.0					
Vit. mixture No. 3.		1.0		1.0						
Choline chloride	0.5	0.5	0.5	0.5	0.5					
Soluble starch			63.5	63.5						
Glucose	63.5	63.5			73.5					
Sesame oil	5.0	5.0	5.0	5.0						
Lard	5.0	5.0	5.0	5.0						

1) 1 kg contained 46.3 g NaCl, 92.0 g NaH_2PO_4, 253.0 g K_2HPO_4, 143.0 g $CaH_4(PO_4)_2H_2O$, 369.0 g Calcium lactate, 70.4 g $MgSO_4$ and 26.3 g KI.

2) 100 g contained 250,000 I. U. vitamin A, 10 mg thiamin nitrate, 15 mg riboflavin, 375 mg ascorbic acid, 20,000 I. U. calciferol, 10 mg dl-α-tocopherol, 100 mg nicotinic amide and 25 mg calcium panthothenate.

3) 100 g contained 250,000 I. U. vitamin A, 10 mg thiamin nitrate, 15 mg riboflavin, 10 mg pyridoxine hydrochloride, 10 γ cyano-cobalamin, 375 mg ascorbic acid, 20,000 I. U. calciferol, 10 mg dl-α-tocopherol, 2 mg vitamin K_3, 100 mg nicotinic amide, 25 mg calcium panthothenate and 5 mg folic acid.

4) Pyridoxine hydrochloride was removed from vitamin mixture No. 2.

5) Purified and peroxide-free sesame oil was used as the source of EFA.

6) From Nissan Syokuhin K. K., containing cholesterol at the 0.17% level.

times with 10 ml of ethyl ether. The ether extract was washed with distilled water to remove chlorides and dessicated with anhydrous sodium sulfate. The ether extract was evaporated in water bath and the residues were dissolved with 10 ml of aceton. An aliquot was pipetted into a small test tube. Aceton was evaporated in water bath of 60°C under current air. 5 ml of 65% sulfuric acid solution was added and heated in water bath of 60°C±1°C for 15 minutes.

After cooling at room temperature for 15 minutes, absorption determinations were made at 3200 Å and 3850 Å by means of BECKMANN DU quartz spectrophotometer. Concentration of bile acids was presented according to GIBB's calculation.

3. Extraction and determination of other lipids in bile

a) Extraction and determination of cholesterol

1 or 2 ml of the drainaged bile was added with 10 volumes of ether ethanol solution (ethyl ether : ethylalcohol 1 : 3 v/v), allowed to stand overnight and warmed in water bath of 50°C to 60°C for 10 minutes, and then filtered. The residues were washed with warmed ether ethanol solution and petroleum ether twice or thrice. Combined ethanol ether extract was then saponificated with 5 ml of 10% KOH ethanol solution at 60°C for 1 hour and extracted with 10 ml of petroleum ether 3 times. The petroleum ether extract was used for determination of total cholesterol in bile.

b) Extraction and determination of phospholipids

An aliquot of the drainaged bile was extracted with ether ethanol solution. The solution was heated on the sand bath with 2.2 ml of conc. surfuric acid and hydrogen peroxide, until colour of the wetashed solution disappeared. Then it was diluted with distilled water until the volume was made up 25 ml. After the solution was added with 1 ml of 33% Na_2SO_4 solution, 10 ml of 2.0% of ammonium morybdate solution and 0.1 g of pure powder of ascorbic acid, it was heated on the steam bath for 5 minutes. After cooling in current water, absorption was determined at 820 mμ with BECKMANN DU spectrophotometer.

Phospholipids were calculated as organic phoshor according to the method which was originated by MORRISON and modified by TANIMURA and HASHIMOTO.

4. Frequency of gallstones

Frequency of the gallstones was presented in percentage as follows : Numbers of the animals with gallstones/numbers of the animals survived more than 8 days after the beginning of the experimental feeding period.

5. Analysis of gallstones

The gallstones were collected, washed with distilled water, desiccated, weighed, crushed, dissolved with chloroform and determined for cholesterol by modified Liebermann-Burchard reaction. The maximal absorption at 640 mμ was measured with BECKMANN spectrophotometer.

6. Histological examination of liver

Liver was examined histologically in some of the experimental animals by means of hematoxylin-eosin double staining.

III. RESULTS

1. Incidence and types of gallstones

Incidence and types of gallstones are presented in a condensed form in Table 2. Distinct difference between carbohydrates was found with respect to the incidence and types of gallstones. When potato starch or soluble starch was chosen as the main source of carbohydrate in Groups 1, 2, 3, 11, 12, 13, 23 and 24, they protected almost completely all the experimental animals against gallstone formation. On the other hand, when the animals received a fat-free diet containing sucrose or glucose as the main source of carbohydrate at 72.0 %, 73.0 % and 73.5 % levels, respectively, in Groups 4, 5, 6, 7, 14, 15 and 25, cholesterol stones occurred at 60 to 90%. Moreover, they were also observed in the animals in Groups 6 and 7 which died of diarrhea and/or prolapsus ani in the earlier period of feeding. These symptoms were mainly observed in case of feeding sucrose. They were prevented by supplementation of the diet with carboxylmethyl cellulose at 1.0 or 2.0% level. No difference between sexes was found in the incidence of gall-

Table 2 Incidence of gallstones

Group No.	Characteristics of diet	Maximal feeding period (days)	Cholesterol stones (%)	Amorphous pigmented stones (%)
1	80.3% potato starch fat-free	95	6.3	0
2	80.3% potato starch fat-free	64	0	5.0
3	63.5% potato starch, 15.0% lard, V. B_6-free	38	0	0
4	73.0% sucrose fat-free	46	80.0	5.0
5	68.0% sucrose, 25.0% casein, fat-free	48	40.0	20.0
6	72.0% sucrose fat-free	15	60.0	0
7	72.0% glucose fat-free	15	33.3	0
8	63.0% sucrose, 10.0% sesame oil	63	6.7	43.3
9	63.0% sucrose, 10.0% lard	52	10.5	36.8
10	66.0% sucrose, 5.0% lard, 2.0% cod liver oil	26	0	100.0
11	74.0% soluble starch fat-free	66	0	0
12	74.0% soluble starch fat-free, V. B_6-free, 0.01% Desoxypyridoxine	66	0	0
13	73.0% soluble starch fat-free, V. B_6-free, 1.0% Sinomin	66	0	0
14	72.0% sucrose, fat-free	35	40.0	20.0
15	72.0% glucose, fat-free	28	80.0	0
16	72.0% lactose, fat-free	15	0	0
17	72.0% dextrin, fat-free	35	20.0	0
18	67.0% dextrin, 5.0% sesame oil	35	0	40.0
19	92.0% casein, carbohydrate and fat-free	15	0	0
20	87.0% casein, carbohydrate-free, 5.0% sesame oil	29	0	0
21	63.5% glucose, 5.0% sesame oil, 5.0% lard	43	66.7	0
22	63.5% glucose, 5.0% sesame oil, 5.0% lard, V. B_6-free	43	50.0	0
23	63.5% soluble starch, 5.0% sesame oil, 5.0% lard	43	0	0
24	63.5% soluble starch, 5.0% sesame oil, 5.0% lard, V. B_6-free	43	0	0
25	73.5% glucose, fat-free	43	90.0	0
26	Rat chow diet	60	0	0

stones. In Group 5, nicotinic acid was added to each 100 g of the diet at 0.25% level. Development of cholesterol stones was slightly inhibited, whereas pigmented stones occurred more frequently than Group 4.

It seemed most likely that sesame oil and lard had a protective property, to some extent, against gallstone formation, especially cholesterol stone formation, respectively. In the animals of Groups 8, 9, 21 and 22, feeding of sesame oil and/or lard at the 5 to 10% levels was associated with a lowered occurrence of cholesterol stones and further with an increased development of amorphous pigmented stones, as compared with the animals fed a fat-free diet. In Group 10, cholesterol stones were completely inhibited, but the amorphous pigmented stones were observed in all the animals. This suggests that cod liver oil also had a protective property against cholesterol stone formation.

At the 72.0% level, lactose kept all the animals free from gallstone formation. They died earlier of unknow cause. None of them could survive more than 15 days after the beginning of the experimental feeding period. Indigestion of α-lactose or deficiency of suitable sugar splitting enzymes in young hamsters may be responssible for this.

Dextrin of which molecule is smaller than that of starch could not also protect the animals against cholesterol stones in a fat deficient state. In some of the animals in Groups 17 and 18, gallstones were developed as the days of the experimental feeding period advanced. In the latter case, sesame oil also protected against cholesterol stones. Amorphous pigmented stones, however, could not be protected by supplementation of the diet with sesame oil only.

None of any types of gallstones occurred in the animals fed a carbohydrate-deficient, high-casein diet, in Groups 19 and 20. When the carbohydrate-deficient diet was supplemented with sesame oil at 5% level, most experimental animls could survive more than 4 weeks after the start of experimental feeding. This suggests that most steps in carbohydrate metabolism, especially glycolysis may be concerned with the mechanisms of gallstone formation.

In the animals of Groups 3, 12, 13, 22 and 24 received a pyridoxine deficient diet, no gallstones occurred in case of feeding starch. In case of feeding glucose, however, cholesterol stones occurred at 50 to 60% levels, though pyridoxine was entirely removed from the diet.

Gallstones presented as cholesterol stones in Table 2 imply pure cholesterol stones (Fig. 1), pure cholesterol stones plus amorphous pigmented stones (Fig. 2), pure cholesterol stones plus amorphous pigmented stones plus mixed stones, amorphous pigmented stones (Figs. 3, 4, 5, 6) and mixed stones (Fig. 7). Amorphous pigmented stones contained little cholesterol.

Discrimination of gallstones were determined microscopically and biochemically. Cholesterol concentration of several types of gallstones was presented in Table 3.

2. Cholesterol, bile acids and phospholipids

a) Cholesterol

Cholesterol concentration of the drainaged bile was considerablly lower in case of feeding starch as compared with the animals received a diet containing sucrose or glucose as the sole source of carbohydrate. In general, sesame oil seemed to decrease to some extent the biliary excretion of cholesterol, regardless of carbohydrates, as seen in Groups 18 and

Table 3 Cholesterol concentration of gallstones

	Types of gallstones	Cholesterol (%)	Diet
1	Pure cholesterol stones	100.0	72.0% glucose, fat-free
2	Pure cholesterol stones	94.0	72.0% sucrose, fat-free
3	Pure cholesterol stones plus amorphous pigmented stones	83.5	72.0% sucrose, fat-free
4	Pure cholesterol stones plus amorphous pigmented stones	80.5	63.5% glucose, 5.0% lard, 5.0% sesame oil
5	Pure cholesterol stones plus amorphous pigmented stones	54.3	72.0% sucrose, fat-free
6	Mixed stones	27.2	72.0% dextrin, fat-free
7	Amorphous pigmented stones	—	72.0% sucrose, fat-free
8	Amorphous pigmented stones	—	63.0% sucrose, 10.0% sesame oil
9	Amorphous pigmented stones	—	63.0% sucrose, 10.0% lard
10	Amorphous pigmented stones	—	66.0% sucrose, 5.0% lard, 2.0% cod liver oil
11	Amorphous pigmented stones	—	80.3% potato starch, fat-free

22.

b) Bile acids

Bile acid concentration was to a great extent decreased in case of feeding sucrose or glucose as compared with feeding of starch, as indicated in Table 4. Supplementation of diets with sesame oil and/or lard revealed an increased biliary excretion of bile acids in case of feeding starch as well as in case of feeding glucose. When pyridoxine was eliminated from the diets, however, the biliary excretion of bile acids also decreased to some extent in both cases of feeding starch and glucose.

c) Phospholipids

In case of feeding starch, phospholipids also showed the same tendency as seen in bile acids. However, in case of feeding sucrose or glucose, phospholipids did not show any significant change, though sesame oil and lard were added to the diets sufficiently. Under a deficient state in pyridoxine, phospholipids also did not show any detectable change in case of feeding glucose. This seemed to indicate that pyridoxine added to the diet was not converted to active form, pyridoxal phosphate, in case of feeding sucrose or glucose. This also suggests that deficiency in pyridoxal phosphate may disturb the biosynthesis of phospholipids in liver, affecting esterification between fatty acids and glycerophosphates, each of which is synthesized to some extent in a fat-deficient state.

d) Ratio of bile acids to cholesterol (B/C) and lipid phosphorous to cholesterol (L/C)

Feeding of lactose showed the highest value in both B/C and L/C. Feeding of sucrose or glucose showed a remarkable decrease of the ratio of total bile acids to cholesterol as compared with feeding of starch. Sesame oil and/or lard showed an inclination to elevate both B/C and L/C. As far as pyridoxine may be concerned, difference was found slightly between Groups 23 and 24, but not found between Groups 21 and 22. This indicates that pyridoxine was not utilized as pyridoxal phosphate in case of feeding glucose, so that the animals showed symptoms referable to pyridoxine deficiency.

In hamsters, B/C and L/C showed the same attitude as observed in rats. Increased

biliary excretion of cholesterol, decreased excretion of bile acids and phospholipids, and decreased ratio of B/C and L/C are all responsible for gallstone formation, especially cholesterol stone formation.

Table 4 Cholesterol, bile acids and lipid phosphorous in the drainaged bile of hamsters fed various diets

Group No.	Diet	Cholesterol (mg/dl)	Bile acids in total (mg/dl)	Lipid P. (mg/dl)	B/C	L/C
11	74.0% soluble starch fat-free	5.5±2.8*	104.2±15.7	36.1±14.4	25.1±9.9	7.5±3.1
14	72.0% sucrose fat-free	9.0±1.0	63.4± 4.5	28.3±11.9	7.1±1.4	3.6±0.5
15	72.0% glucose fat-free	9.7±3.2	63.6± 3.1	25.1±12.6	7.7±2.4	2.4±0.7
16	72.0% lactose fat-free	3.8±0.8	124.1±13.6	52.5± 8.8	33.7±3.2	16.8±3.6
17	72.0% dextrin fat-free	9.9±4.2	102.1±19.9	42.9±18.0	10.9±2.8	5.5±3.2
18	67.0% dextrin, 5.0% sesame oil	5.4±1.0	109.5±30.3	57.6±20.9	22.2±7.8	9.3±2.8
20	87.0% casein, 5.0% sesame oil	9.6±1.6	139.1±38.1	31.8± 7.4	15.5±5.3	6.0±2.3
21	63.5% glucose, 5.0% sesame oil, 5.0% lard	13.6±9.6	85.2±13.0	30.2± 8.9	12.4±8.9	4.7±3.5
22	63.5% glucose, 5.0% sesame oil, 5.0% lard, V. B_6-free	6.6±0.8	78.6± 6.4	30.4± 9.8	12.0±0.5	4.2±1.6
23	63.5% soluble starch, 5.0% sesame oil, 5.0% lard	6.5±2.3	140.8±67.3	41.5±13.6	20.9±3.9	7.0±2.4
24	63.5% soluble starch, 5.0% sesame oil, 5.0% lard, V. B_6-free	6.0±2.6	114.0±42.8	27.0±15.5	19.3±4.5	4.6±2.1
25	73.5% glucose fat-free	14.3±7.8	63.3±17.2	27.9±11.1	5.9±2.2	2.4±0.7
26	rat chow diet	8.9±1.7	122.8±38.7	49.8±10.8	13.4±2.0	5.9±1.9

* : Standard deviation

3. The action of intestinal flora and vitamins

When potato starch was chosen as the main source of carbohydrate in a fat-free diet (Group 1), most animals could survive longer after the beginning of the experimental feeding period, though pyridoxine, biotin, inositol, folic acid and vitamin K were all removed from the diet. This indicates that these vitamins are synthesized by the action of the intestinal flora so sufficiently as to meet demand of the host.

4. Histological examination of liver

Degeneration of liver cells, proliferation of interstitial tissues, infiltration of lymphocytes and leucocytes and stagnation of bile were observed to a lesser grade in some of the experimental animals. Thus variations of the dietary composition gave no significant influence upon the obligatory factors initiating gallstones, affecting directly or indirectly liver functions in hamsters.

IV. DISCUSSION

The chief end-products of cholesterol degradation are bile acids, although cholesterol is also converted into sex hormones and adrenocortical hormones. It has been fairly well demonstrated that feeding of a fat-deficient diet was associated with a marked decrease of the concentration of bile constituents, as compared with the animals fed a fat diet. This is the reason why the animals cannot synthesize EFA in a fat-deficient state and little

cholesteryl arachidonate may be produced. Cholesterol esterified with tetraenoic acids, especially arachidonic acid is more rapidly metabolized to bile acids than cholesterol esterified with the other fatty acids. Therefore, the marked decrease of biliary excretion of bile acids may be attributed to the inhibited synthesis of cholesteryl arachidonate in liver, when the experimental animals received a fat-deficient diet.

In hamsters fed a fat-deficient diet containing starch as the main source of carbohydrate, bile acids and lecithin which was presented as lipid phosphorous decreased to some extent, but any type of gallstones did not occur. In those fed a fat-deficient diet containing sucrose or glucose as the main source of carbohydrate, however, a marked decrease of both bile acids and lecithin was found, being associated with an elevated biliary excretion of cholesterol and highly occurrence of cholesterol stones.

Difference between carbohydrates was found with respect to changes in bile constituents as well as incidence of gallstone formation. This may be attributed to the following factors.

1) The hepatic synthesis of bile acids may be regulated by the action of the intestinal flora of which multiplication may also be affected by difference between carbohydrates chosen as the source of carbohydrate in the basal fat-free diets. Furthermore, the synthesis of bile acids appears to be regulated by the concentration of bile acids in the portal blood. According to the experiments of PORTMAN, GUSTAFSSON and DANIELSSON, a number of metabolites formed from the excreted bile acids by the action of the intestinal flora are not reabsorbed under the physiological conditions and excreted in feces. Thus, the decreased concentration of bile acids in the portal blood promotes the hepatic synthesis of bile acids. This suggests that feeding of sucrose or glucose, each of which is almost completely absorbed at the upper part of the intestines, leads to inhibit multiplication of the intestinal flora for lack of nutrients at the lower part of the intestines, leading simultaneously to increase the hepatic pool size of bile acids which in turn inhibits the hepatic synthesis of bile acids. Therefore, daily production and then biliary excretion of bile acids may be decreassed in case of feeding sucrose or glucose, as compared with the animals fed polysaccharides.

2) The actions of several kinds of vitamins formed by the intestinal flora must be noted. Deficiency and/or a decrease of activity in pyridoxine may give a great influence upon EFA metabolism, leading to the inhibited hepatic synthesis of cholersteryl arachidonate which decrease the hepatic synthesis of bile acids. HOLMAN et al. reported that conversion of linoleic acid to arachidonic acid was promoted to a great extent by pyridoxine. Complete deficient state in pyridoxine was not produced in the experimental animals in case of feeding starch, though pyridoxine was entirely removed from the diet, since pyridoxine was formed by the action of the intestinal flora, preventing the development of symptoms referable to pyridoxine deficiency. It is well known that difference in activities was found in relation to availability of vitamins in vivo. As far as pyridoxine is concerned, therefore, it is questionable whether or not pyridoxine added in the diet was converted to the active form, being utilized as pyridoxal phosphate in case of feeding sucrose or glucose.

From this point of view, the activities of vitamins should be taken into consideration, when experiments are designed to research the effects of vitamins which are supplied to

some extent by the intestinal flora to meet demand of the host, such as pyridoxine, biotin, inositol, folic acid and vitamin K.

3) When sucrose or glucose is chosen as the main source of carbohydrate in a fat-free diet, cholesterol stones are formed in the earlier period of feeding. According to the experiments reported by DAM et al., glycosuria was not demonstrated, though the animals intook and absorbed a large amount of glucose. This suggests, therefore, that the large amount of absorbed glucose may be quickly metabolized through hexosemonophosphate shunt.

In rats, absorption of sucrose or glucose from the stomach usually has not been observed. In hamsters the opinion may also be the same as rats. It is apparent from the experiments that sucrose or glucose may be quickly absorbed from the upper part of the small intestine. The increased concentration of carbon dioxide in the expired air soon after ingestion of sucrose or glucose appears to demonstrate that the large quantities of absorbed sucrose or glucose may be quickly splitted via hexosemonophosphate shunt, bringing about urgent metabolic changes in liver. On the other hand, polysaccharides are usually absorbed and metabolized more slowly than sucrose or glucose and other easily absorbable carbohydrates. Thus, in case of absorption of polysaccharides, liver escape such urgent metabolic changes as seen in case of sucrose or glucose. In the case of absorption of glucose, the large amount of absorbed glucose requires departure of glycolysis via hexosemonophosphate shunt which in turn produce the unbalanced conditions for some proper enzymes in liver. For instance, TPN-TPNH and DPN-DPNH enzyme systems are involved in the process. They are counteracting each other, therefore, the increased activity of TPN-TPNH system inhibits DPN-DPNH system, leading to an increase in the endogeneous synthesis of cholesterol and further a decrease in the hepatic synthesis of bile acids.

The development of amorphous pigmented stones in which cholesterol content was very lower, has been more frequently encounted in case of supplementation of diets with unsaturated fatty acids, sesame oil and cod liver oil than in case of supplementation with saturated fatty acids, lard. Feeding of polysaccharides plus fats also protected all the experimental animals against gallstones completely. Feeding of monosaccharide or disaccharide, excluding lactose was associated with the regular occurrence of cholesterol stones. When supplemented with unsaturated fatty acids, however, white or light yellow cholesterol stones disappeared almost completely and amorphous pigmented stones occurred at the rate of ca. 50%. This indicates that unsaturated fatty acids, for example, sesame oil in which EFA is contained no less than 50 %, has a protective property against gallstones, especially cholesterol stones. This also suggests that a great quantity of cholesterol which was synthesized through the obligatory hexosemonophosphate pathway may be easily converted into bile acids by esterification with EFA, leading to an increase in the biliary excretion of bile acids and in the ratio of bile acids to cholesterol. Then the capacity of bile keeping cholesterol in solution is increased, protecting against precipitation of cholesterol and formation of cholesterol stones.

V. SUMMARY AND CONCLUSION

Several alimentary effects upon gallstone formation in hamsters have been discussed.

The following conclusions were obtained.

1) In the animals fed a fat-deficient diet, gallstones which were consisted chiefly of cholesterl occurred more frequently than those fed a fat diet.

2) Difference between carbohydrates chosen as the main source of carbohydrate in the various synthetic diets was found with respect to the incidence and characteristics of gallstones.

3) Supplementation of the diets with fats had to some extent a protective property against gallstone formation. Unsaurated fatty acids, cod liver oil and sesame oil, especially the latter containing much EFA protected against cholesterol stones whereas the development of amorphous pigmented stones were not protected.

4) In the animals fed a fat-deficient diet from which such vitamins produced by the action of the intestinal flora as pyridoxine, biotin, inositol, folic acid and vitamin K were all entirely removed, symptoms referable to their deficiency were not so completely developed that most animals could survive over longer period without gallstones, when starch was chosen as the main source of carbohydrate.

5) In the drainaged bile of hamsters, cholesterol, bile acids and phospholipids were determined, respectively. In Groups accompanied with the highly incidence of gallstones, an increase in cholesterol concentration, decreases in bile acid and phospholipid concentration, and decreases in B/C and L/C were observed. In those without gallstones, however, these changes were not observed.

6) These changes also seemed to be inducements for gallstone formation in hamsters as well as in rats.

7) Thus, in hamsters, the experimental hypothesis was established that EFA deficiency and/or metabolic disturbances in EFA might affect directly cholesterol degradation to bile acids and disturb the biosynthesis of lecithin, leading to the decreased biliary excretion of bile acids and lecithin which in turn weakened the stability of bile to keep cholesterol in solution so that cholesterol began to crystallize, precipitate and then cholesterol stones occur.

8) Analysis of the gallstones revealed that white or light yellow stones contained cholesterol over 80%, but green or dark brown amorphous pigmented stones contained little cholesterol.

9) Histological examination of liver showed pathological changes only slightly in some of the experimental animals. Disturbances in liver functions seemed to have no significant influence upon gallstone formation.

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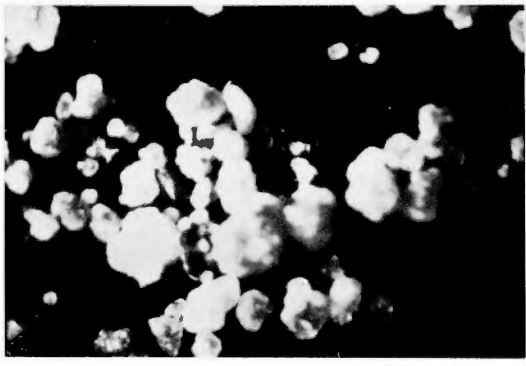

Fig. 1 White Stones from a hamster fed a fat-free diet containing sucrose as carbohydrate source (Group 4). They were found in the gallbladder at operation after 46 days of feeding. Magnification 20 times.

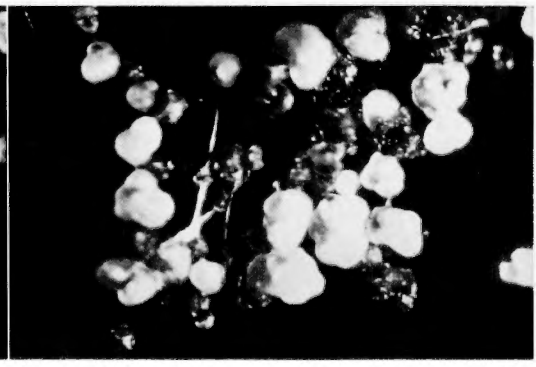

Fig. 2 Light yellow stones and dark brown stones from a hamster fed a fat-free diet containing sucrose as the source of carbohydrate (Group 4). They occurred together in the gallbladder at operation after 46 days of feeding. Magnification 20 times.

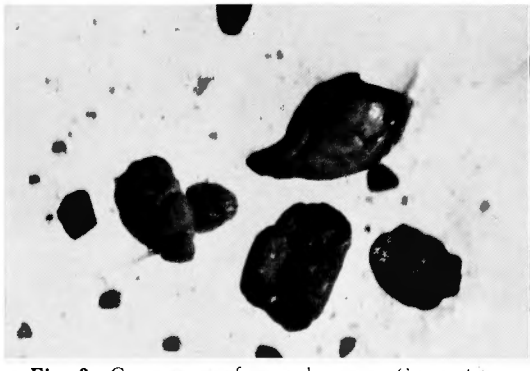

Fig. 3 Green stones from a hamster (Group 4.) died of diarrhea after 19 days of feeding. One of them projected partly through the neck of the gallbladder into ductus cysticus. Magnification 20 times.

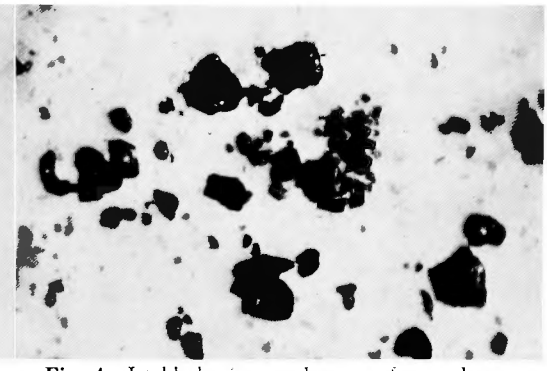

Fig. 4 Jet black pigmented stones from a hamster receiving a fat diet supplemented with sesame oil. (Group 8.) They occurred after 25 days of feeding. Magnification 20 times.

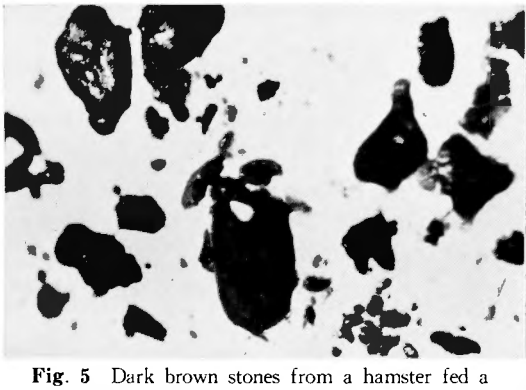

Fig. 5 Dark brown stones from a hamster fed a fat diet sapplemented with lard. (Group 9.) They were found in the gallbladder at autopsy after 28 days of feeding. Magnification 20 times.

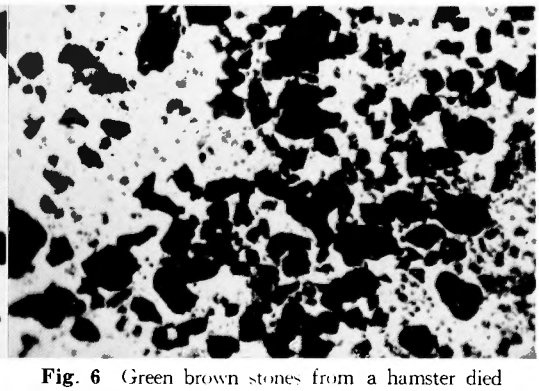

Fig. 6 Green brown stones from a hamster died on 12th day after the start of feeding. (Group 10.) Magnification 20 times.

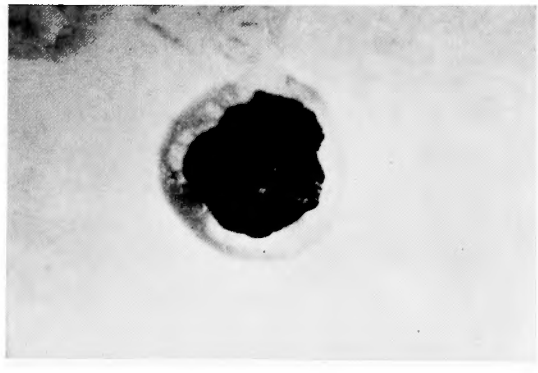

Fig. 7 One grey large stone from a hamster fed a fat-free diet containing dextrin as carbohydrate source. It was found in the gallbladder at operation after 35 days of feeding. Magnification 10 times.

和文抄録

胆石形成に関する実験的研究

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塩田隆三

我々の教室では，日笠等はコレステロール代謝に関する不可欠脂酸の特殊生理学的作用に就いて探究し，副腎に於て，コレステロールとエステル結合した不可欠脂酸，特にTetraenoic acidはコレステロールのGlucocorticoidへの代謝を直接促進し，副腎皮質機能に重大な影響を有するという事を確認するに至つた．一方，胆汁酸はコレステロールの主終末代謝産物であり，肝に於ける胆汁酸合成過程においても，亦コレステロールが不可欠脂酸とエステル結合する事が必要であり，不可欠脂酸の欠乏，乃至はその代謝障害の存在する場合には，胆汁酸の生合成障害，胆汁中への分泌能の低下を招来するという事が充分憶測され得る．又レシチンの生合成も不可欠脂酸代謝と密接な関係を有する事が判明した．かくして，不可欠脂酸の欠乏，乃至その代謝障害はコレステロールの胆汁酸への代謝，並びにレシチンの生合成を障害し，胆汁中の胆汁酸及びレシチンの濃度の低下，胆汁酸対コレステロール（B/C），レシチン対コレステロール（L/C）の比の減少を招来し，これらがコレステロールに対する胆汁の不安定化の誘因となり，コレステロールの析出，沈澱，結石形成を招くという実験的仮設が構立されるに至つた．然しながら，上記実験はすべてウイスター系雄性ラットを使用したものであり，ラットには胆嚢がなく，実験的仮説の実証のためには，胆嚢を有する試獣が選ばれなければならない．胆汁組成がラットに近似したハムスターを選び，種々の合成食飼にて飼育し，実験的に胆石を作成することに成功し，更に，ドレナージ胆汁中のコレステロール，胆汁酸，レシチンの分析，胆石の分析，肝の組織学的検索を行ない，次のような結果を得た．

1） 無脂肪食飼育に際しては，脂肪食飼育の場合よりも高率に，コレステロール石の発生を認めた．

2） 胆石の発生頻度及びその特性に関して，合成食飼中の主糖質補給源間に著明な差異の存在する事を認めた．

3） 食飼に添加された脂肪は，ある程度，胆石形成を防止する．不飽和脂酸，特に不可欠脂酸を豊富に含有するゴマ油には，コレステリン石発生予防の性質が認められたが，色素石の発生は防止されなかつた．

4） 腸内細菌により合成され得るピリドキシン，ビオチン，イノシトール，葉酸，ビタミンKの如きビタミンを，すべて欠除した無脂肪食で飼育された試獣では，糖質補給源として，澱粉が使用される場合には，これらビタミンの欠乏症状は惹起され難く，試獣は長期間の生存に耐え得た．

5） 高率に胆石発生を認めた群では，ドレナージ胆汁中のコレステロールの増加，胆汁酸及び，レシチンの減少，更に，B/C及びL/Cの比の低下が認められた．しかし無石群では，これらの変化は認められなかつた．

6） 此種の変化は，ラットと同様にハムスターに於ても，亦胆石形成の誘因であると思われる．

7） かくして，不可欠脂酸の欠乏，乃至その代謝障害はコレステロールの胆汁酸への移行に直接影響を与えると同時に，レシチンの生合成も障害し，胆汁酸及びレシチンの胆汁中への分泌を抑制し，これらが胆汁のコレステロールに対する不安定化を招来し，ために，コレステロールが析出，沈澱し，コレステロール石の形成を来すという実験的仮説が，ハムスターに於て立証されたことになる．

8） 胆石分析の結果，白色乃至薄黄色石は，コレステロールを80%以上含有し，緑色乃至暗褐色石はコレステロールをほとんど含有しない事が判明した．

9） 肝の組織学的検索に際しては，肝の病理学的変化は極く軽度であり，胆石形成には，肝機能障害は特別な意義を有しないものと思われる．